ISSN: 0974-5343
IJMST (2017), 7(2):10-17

Fungal infection in blood culture negative neonates, in NICU

Mona Mohiy Eldeen, MD; Amal mohamed Sayed, MD; and Dalia Kadry Ismaiel, MD
Clinical and Chemical Pathology Department, Faculty of Medicine, Cairo University, EGYPT
imaging70@gmail.com, mmohiedden@yahoo.com, hoelwan@gmail.com

Abstract:
Candida has been become an increasingly important in neonates hospitalized in neonatal intensive care unites(NICUs). *Candida* species are recognized as leading pathogens in the neonatal intensive care unit (NICU) for infections occurring after the third day of life This was a prospective study included 79 out of 1742 (4.5%) Septicemic neonates admitted to Neonatal Intensive Care Unit - Cairo university specialized pediatric hospital (CUSPH), during the period from August 2009 to August 2010 and having an evidence of sepsis. In this approach we performed amplification of a sequence of Candida most commonly involved in human infection, by employing universal fungal primers, followed by species specific primer for C.albicans being the most clinically important species, and we compared our method with the conventional diagnostic and identification methods for early detection of candidemia. They participant were broadly divided into two groups.
(i) Candidemic patients (n _ 73): They had at least two positive Candida blood cultures. (ii) Patients with suspected candidemia, (n _ 6); they were admitted to the neonatal intensive care unit for more than 1 week., all patients of this group were tested two to three times by blood culture and were always negative, they were treated with broad spectrum antibiotics for a minimum of 1 week and developed fever inspite of prolonged antibiotic therapy suggesting Candida infection.
In addition, 10 apparently healthy individuals with no complaints of Candida infection were included as controls.
The comparative results of blood culture and the PCR for the detection of candida spp. showed that among the 79 blood broth specimens; 73 cases (92%) were blood culture positive for candida, while 75 (95%) were positive by PCR using blood broth specimens, with two more cases positive for candida despite being repeatedly culture negative. Among 79 blood broth specimens, 9 cases(11%) were positive by Germ-tube test versus 43 cases (54%) positive by PCR for C.albicans.
Among 27 whole EDTA blood specimens 6cases(22%) were positive by Germ-tube test versus 19 cases (70%) were positive by PCR using C.albicans specific primers . Amplification of the product of the PCR with species-specific primers corresponding to the ITS2 sequences from
C. albicans resulted in specific amplification of single DNA products of the expected sizes .
Key words: NICU, Vertical transmission, Candida, Antifungal, PCR, candidemia, EDTA-blood specimens

Introduction:
Bloodstream infections are major causes of morbidity and mortality and represent the eighth leading cause of infant death [1]. Candidemia is the second most common nosocomil bloodstream infection in very low weight neonates and children [2, 3]. Mortality rates associated with candidemia remain significant despite use of advanced therapeutic approaches [3].Although most data collected are from adults, availabl evidence indicates that Candida species cause ahigh proportion of bloodstream infections among children, especially neonate because of their immature immune system and the invasive supportive care they require[Candida species colonize up to 60% of low-birth-weight neonates during their first month in the neonatal intensive care unit (NICU)). Exposure of newborns to fungi is a complex problem and results from both horizontal and vertical transmission. Prolonged use of broad specrum antibiotics was strongly associated with candidemia. [5&6]. Bloodstream infections due to Candida have risen to become the fourth-most-frequent cause of septicemia, with an attributable mortality rate of about 50% despite the availability of antifungal therapy. [7]

To reduce mortality rates for patients with invasive candidiasis, early initiation of antifungal drug therapy is critical. However, diagnosis remains difficult, since the only sign of infection may be a prolonged fever that is refractory to antibacterial treatment. [8]. In absence of pathognomonic signs or symptoms of systemic candidiasis, the diagnosis is based on isolation of candida species from blood cultures or tissue

ISSN: 0974-5343
IJMST (2017), 7(2):10-17

biopsy specimens. Blood culture remains the accepted 'gold standard' method for diagnosis of candidiasis. However, in some settings, culture can fail to detect Candida spp. in more than 50% of patients with chronic disseminated candidiasis especially in early stages of the disease. [7]. moreover it is time consuming and often labor intensive even with an optimal blood culture system and since it has been shown that the prognosis is better when treatment is started early, it is usually recommended that antifungal therapy be started as soon as a strong suspicion of systemic candidiasis exists On the other hand, such empiric antifungal therapy may be unnecessarily toxic and costly, and it may increase the selective pressure towards more-resistant Candida species[8] .Thus, efforts have been made to develop more- rapid, sensitive, and specific test to aid in the diagnosis of the disseminated yeast infection. The limitations of classical diagnostic methods for invasive fungal infections (IFIs) have led to considerable progress in non-culture diagnostics, particularly in genomic amplification methods. [9]. DNA-based diagnostic tests not only are sensitive and specific but also have the potential to decrease the time taken for the laboratory identification of pathogens that are slowly growing or difficult to culture. Such tests may detect nonviable and nonculturable cells as well as viable cells .The polymerase chain reaction offers an alternative approach for the specific and rapid detection of pathogenic yeast. Procedures suitable for identifying Candida directly from clinical specimens are now emerging. They are based on genomic amplification methods and seem to detect and identify a large number of related species of pathogenic yeast using distinct target DNA sequences [9]. A rapid identification of yeasts provides timely in information for patient management, which allows effective and early antifungal therap.

Patients And Method:

Subjects: This was a prospective study included 79 out of 1742 (4.5%) Septicemic neonates admitted to Neonatal Intensive Care Unit - Cairo university specialized pediatric hospital (CUSPH), during the period from August 2009 to August 2010 and having an evidence of sepsis. Our 79 participants were not improving on intra venous antibiotics for 7days or more. They were broadly divided into two groups.

(i) Candidemic patients (n _ 73): They had at least two positive Candida blood cultures. (ii) Patients with suspected candidemia, (n _ 6). They were admitted to the neonatal intensive care unit for more than 1 week.all patients of this group were tested two to three times by blood culture and were always negative,they were treated with broad spectrum antibiotics for a minimum of 1 week and developed fever in spite of prolonged antibiotic therapy suggesting Candida infection.

In addition, 10 apparently healthy individuals with no complaints of Candida infection were included as controls.

Blood culture broth specimens were obtained from all 79 participants in addition to the 10 control subjects (Total: 89). Of these, 37 participants had a whole EDTA-blood specimen including the 10 controls, submitted for analysis simultaneously with the blood broth specimens.

The data relating to the whole-EDTA blood specimens were not included in the description of the original trial, as it was not principally intended to evaluate this specimen type. The present report compares PCR performance in whole-EDTA blood specimens from this subset of 37 participants with that in the paired blood broth specimens already reported as part of the original trial.

In the 37 paired specimens, 23 pairs of specimens were submitted from participants with Laboratory-proven candidemia, whilst 4 pairs were from participants categorized as patients with suspected candidemia and 10 pairs of controls. All specimens were kept frozen at -80 C until extracted.

Routine laboratory investigations included:
CBC, CRP, blood culture and antibiotic sensitivity, serum bilirubin, blood gases, urine analysis, and CSF analysis when indicated.

Microbiological investigations:

Blood cultures were obtained from peripheral veins by sterile technique, specimens were inoculated into BACTEC Peds Plus/F (Becton-Dickinson, Sparks, MD, USA) cultures bottles. All cultures were monitored using an automated culture system. Passages to blood agar and Sabouraud dextrose agar were performed. The isolated yeasts (*C. albicans* and non-*albicans* species) were presumptively identified as *C. albicans* using morphological criteria (germ tube and chlamydospore formation). Bottles flagged as positive whose contents were negative for any microbial pathogen by Gram stain

ISSN: 0974-5343
IJMST (2017), 7(2):10-17

returned back to the BACTEC instrument for continued monitoring. Bottles that subsequently contained bacterial pathogens by culture were excluded from further evaluation. All bottles that didn't flag positive were subcultured on SDA| agar and on sheep blood agar on 5th day of incubation at date of culture termination. The germ tube positive isolates were provisionally identified as C.albicans species, and then confirmed as C.albicans using conventional laboratory methods including subculture on differential rice agar and biochemical reaction including urea agar medium, sugar fermentation and sugar assimilation media. Blood culture bottles and whole EDTA blood samples of the isolates that were proved to be candida albicans either by germ tube or biochemical reactions and those that were identified as Candida non- albicans by any of the conventional methods, were kept at -80C for further confirmation using PCR assay.

Molecular technique:
Extraction of Candida DNA from both positive control and broth samples (C. albicans ATCC 2091 was supplied from depaertment of Microbiology, faculty of veterinary medicine cairo university).

A modified QIAamp (QIAGEN, Hilden, Germany) DNA extraction protocol was used. A small amount of culture was taken from the culture plates and boiled for 10 min in 100 ul of freshly prepared 25 mM NaOH and 0.5% sodium dodecyl sulfate. After cooling and neutralization with 100 |xl of 25 mM HCl, 200 u,l of buffer AL (QIAamp kit) was added, and the suspension was boiled again for 10 min. Then, the QIAamp protocol was performed according to kit instructions.

DNA from the rat lung was prepared as described for clinical specimens. The amount of DNA was quantitated spectro-photometrically sambrook, and the DNA was stored in aliquots at -20°C.
Extraction from broth samples; were centrifuged at 3000 rpm for 10 min then the pelette were suspended and boiled for 10 min in 100 ul of freshly prepared 25 mM NaOH and 0.5% sodium dodecyl sulfate. After cooling and neutralization with 100 ul of 25 mM HCl, proceed as described in QIAamp kit.

Processing of blood samples for PCR:
Extraction of DNA from patient whole EDTA-blood:
DNA was extracted from 3 ml EDTA-treated whole blood according to the method published by Lo¨ffler et al. (1997) as this method has been extensively used and described in the literature for extraction of fungal DNA from EDTA-treated blood. An extraction control was processed in parallel with each batch of samples.

Oligonucleotide primers and PCR.
The fungus-specific universal primers ITS1 (5' TCC GTA GGT GAA CCT GCG G CCT 3') and ITS4 (5' CCT CCG CTT ATT GAT ATG C 3'), were designed by Hendolin et al 2000 according to sequence published by White et al; 1990. They were used as outer primers to amplify the intergenic transcribed spacer regions. The fungal ITS region, comprising ITS1, the 5.8S rRNA gene, and ITS4, was amplified with previously described universal primers. ITS1-ITS4 sequences derived from GenBank with position accession number of 1769–1787 (M27607) and 820–838 (D89886) respectively with a product size of 848 bp. Specific inner primers were designed for C. albicans, on the basis of the ITS1–ITS4 sequences derived from GenBank (accession number of L47111)

PCR amplification:
A 50-µl reaction mixture contained x µl sample DNA , containing 1 µg of extracted DNA (either from EDTA blood or blood broth culture) and 100 ng of positive C.albicans. 25 picomol of each outer and inner primer, 2 ul of dntp mixture and 5 ul of 10X PCR Gibco BRI., Gaitherburg, Md , usa(10 mM Tris-HCl [pH 8.3 at 25°C], 50 mM KCl) supplied with 1.5 mM MgCl2 (Gibco BRI., Gaitherburg, Md.), 2.5% dimethyl sulfoxide (Amersham Pharmacia Biotech, Uppsala, Sweden), and 2 U of AmpliTaq Gold DNA polymerase(2 U, Gibco BRI., Gaitherburg, Md). Reaction mixtures were prepared from prealiquoted reagents in a laminar-flow hood dedicated for PCR, using aerosol-resistant micropipette tips. Each PCR assay included a positive control containing 0.5 ng of purified DNA of one of the fungal isolates and at least two blanks with reagents only. Samples and controls were transferred to PTC200 thermal cycler (MJ research , USA). The reaction profile was as follows: 5 min of initial denaturation at 94°C, followed by 39 cycles of 96°C for 1 min, 55°C for 45 s and 72°C for 1 min and a 10-min final extension at 72°C. The PCR for specific amplification of C.albicans was performed under the same previously described conditions except for the annealing temperature which was specifically 66 C for C.albicn.

ISSN: 0974-5343
IJMST (2017), 7(2):10-17

Results

Study population;

Blood culture broth specimens were obtained from all 79 participants in addition to the 10 control subjects (Total: 89). Of these, 37 participants had a whole EDTA-blood specimen including the 10 controls, submitted for analysis simultaneously with the blood broth specimens.

Control cases for standardization of PCR;

All control cases (10 broth and 10 EDTA specimens) were negative for C.albicans by both culture and PCR techniques. They were clinical and laboratory normal persons).

Blood broth specimens

The comparative results of blood culture and the PCR for the detection of candida spp. showed that among the 79 blood broth specimens; 73 cases (92%) were blood culture positive for candida, while 75 (95%) were positive by PCR using blood broth specimens, with two more cases positive for candida despite being repeatedly culture negative. The incidence of candida among studied cases was 4.3%.

The PCR amplification of rDNAs using universal fungal primers (CTSF and CTSR) resulted in amplification of a single DNA fragment of the expected size (Fig. 1).

Whole blood EDTA specimens

Same 23 specimens which were blood culture positive were also positive by PCR using universal panfungal primers in whole blood EDTA specimens and in blood broth specimens.

The 4 cases (15%) that were negative by blood culture were also negative by PCR in whole
EDTA blood specimens .(Table 2).

Comparison of Germ-tube test versus PCR for detection of C.albicans;

Among 79 blood broth specimens, 9 cases(11%) were positive by Germ-tube test versus 43 cases (54%) positive by PCR for C.albicans. Among 27 whole EDTA blood specimens 6cases(22%) were positive by Germ-tube test versus 19 cases (70%) were positive by PCR using C.albicans specific primers Table 3. Amplification of the product of the PCR with species-specific primers corresponding to the ITS2 sequences from C.albicans resulted in specific amplification of single DNA products of the expected sizes (Fig. 2).

Discussion:

Early detection of bloodstream infections (BSI) is crucial in the clinical setting. Blood culture remains the gold standard for diagnosing BSI. Molecular diagnostic tools can contribute to a more rapid diagnosis in septic patients[9].Existing diagnostic methods using Candida blood culture, antigen, or antibody detection are lacking sensitivity and specificity .DNA based diagnostic tests not only are sensitive and specific but also have the potential to decrease the time taken for the laboratory identification of pathogens that are growing slowly or difficult to culture. Therefore, earlier detection and identification would facilitate a prompt and appropriate treatment to decrease the fulminant and rapidly fatal outcome of invasive candidiasis.[10]

This study asseses the clinical performance of PCR for detection of Candida DNA directly from EDTA-blood specimens, even with culture negative cases, and we compared our method with the conventional diagnostic and identification methods for early detection of candidemia.

According to our results the microbiological incidence of candida was 73 out of 1742 positive cases by conventional blood culture (4.2%) throught one year. In other studies by Leibovitz *2012* the incidence of candida in blood culture was 12.2% in very low birth weight infants, the infection in these patients are associated by high onst of morbidity and mortality[11].

The diagnosis of candidemia continues to be a major challenge for clinicians because patients commonly present with nonspecific symptoms. This is especially true in very low birth weight neonates; 30% of all cases of neonatal systemic candidiasis are not diagnosed until autopsy[12]. The current methods for diagnosing candidemia rely on culturing yeasts from blood .There are problems inherent in using this method, because culture often requires blood volumes not easily obtained from a very low birth weight infant, and several days are needed before the organism is detected and identified. In fact, 40 to 60% of all blood cultures remain negative for Candida species, despite a widespread visceral infection of a fungal nature[13].

The comparative results of blood culture and the PCR for the detection of candida spp. showed that among the 79 blood broth specimens of clinincal suspicious cases; 73 cases (92%) were blood culture positive for candida, while 75 (95%) were positive by PCR for

candida using blood broth specimens, resulting into two more cases positive for candida by PCR despite being repeatedly culture negative.

This result is agreed with several other studies where PCR has been confirmed to be more sensitive than conventional culture methods in the diagnosis of candidaemia .

It has been demonstrated that PCR can be performed either on whole-blood samples or on broth samples [14& 15]. However, the efficiencies of the same PCR assay applied simultaneously to broth or whole blood have might not be equivalent, since the DNAs of candida present in the two types of samples are probably different in origin. Indeed, only free template DNA should be detectable in broth samples, since fungal cells are eliminated by centrifugation without having been lysed to release intracellular DNA . By contrast, when whole-blood samples are used, both free DNA and intracellular DNA could be present when the sample is drawn from the patient. However, because of the presence in blood of PCR inhibitors, such as hemoglobin, a decontamination step, including lysis of blood cells and washing, is performed first. These steps probably eliminate free Candida DNA, leaving intracellular Candida DNA as the sole possible target for the PCR assay.. Thus, depending on the sample used, the origin of the detected DNA probably varies. In study by Tomer etal 2011 PCR positivity rates among patients with proven or probable invasive candidiasis(IC) were 85% (78 to 91%), while blood cultures were positive for 38% (29 to 46%)[16]. They conclude that direct PCR using blood samples had good sensitivity and specificity for the diagnosis of IC and offers an attractive method for early diagnosis of specific *Candida* sppIts effects on clinical outcomes should be investigate[16].

Same 23 specimens which were blood culture positive were also positiv by PCR using universal panfungal primers in whole blood EDTA specimens and in blood broth specimens. The 4 cases (15%) that were negative by blood culture were also negative by PCR in whole EDTA blood specimens,while 2 cases out of these 4 blood culture negative cases were candida positive by PCR in blood broth specimens.

In previously published clinical studies evaluating the sensitivity of PCR assays in the diagnosis of candidemia, where samples for PCR were drawn at the same time as blood cultures and were frozen, and only those yielding positive cultures were later assayed by PCR[17]. In one study, Garczewska 2002 the sensitivity of a C. albicans- and C. glabrata-specific nested PCR performed on whole blood was 90% (19 of 21 positive blood cultures tested) [18].

In another study by Maaroufi,2003 in which a C.albicans-specific probe was used for the detection of Candida DNA amplified from whole blood, all samples tested were positive by PCR, but they all had Candida counts equal to or greater than 20 CFU/ml [19]. In another study by Bougnoux et al 1999, the only four false-negative PCR results on whole blood were from samples with Candida cell counts below 10 CFU/ml. In another study by Fredricks & Relman; 1998, they concluded that blood culture SPS in blood culture broth is a potent inhibitor of PCR , but it can be removed by benzyl alcohol extraction protocol which improves PCR performance . In accordance with our results Fujita et al, confirmed complete agreement between conventional diagnostic and PCR methods for identification of Candida.[20.Candida albicans were diagnosed in 9 samples from 79 blood broth samples by Germ tube test (11%), while there was 43 cases (54%) were detected by PCR specific primers for C.albicans.

Among 27 whole EDTA blood specimens, 6 cases(22%) were positive by Germ tube test versus 19 cases (70%) were positive by PCR using C.albicans specific. the germ-tube test is suitable if a laboratory aims only at separating the numerous isolates of Candida albicans from the less frequently occurring non-albicans yeasts. Other aspects must therefore be taken into account by laboratory personnel regarding the decision of whether a test is suitable for the given situation.

The germ-tube test is the cheapest test, but it is time consuming and laborious (100 yeast cells should be examined before the test is declared negative). Experience is needed for correct recognition of germ tubes.[21].

ISSN: 0974-5343
IJMST (2017), 7(2):10-17

Table (1): Clinical samples:

Culture result / Sample type	Candida Positive	Candida Negative	Controls	Total
EDTA - blood	23	4	10	37
Broth	73	6	10	89

Table 2: Comparison of blood culture versus pcr in both EDTA and broth samples.

Specimens	BACTEC culture	PCR	% Agreement
Blood broth specimens	73	75	97%
Whole EDTA blood specimens	23	23	100%

Table 3: Comparison of Germ tube test versus PCR for detection of C.albicans in both EDTA and broth samples.

Specimens	Germ-tube test	PCR	% Agreement
Blood broth specimens	9	43	20%
Whole EDTA blood specimens	6	19	31%

Table 4:Rresults of blood cultures versus pcr in broth samples and in EDTA blood sampls.

Sample	Broth(89)		EDTA(37)	
	Cases (79)	Control (10)	Cases (27)	Control (10)
Culture results (positive)	73	------	23	----
(Negative)	6	10	4	10
PCR results (panfungal) (positive)	75	0	23	0
(Negative)	4	10	4	10
C.albicans (Germ tube) Positive	9	---	6	---
Negative	64	10	17	10
C.albicans (PCR) Positive	43	--	19	---
Other fungal infection (Candida spp)	32	10	4	10

ISSN: 0974-5343
IJMST (2017), 7(2):10-17

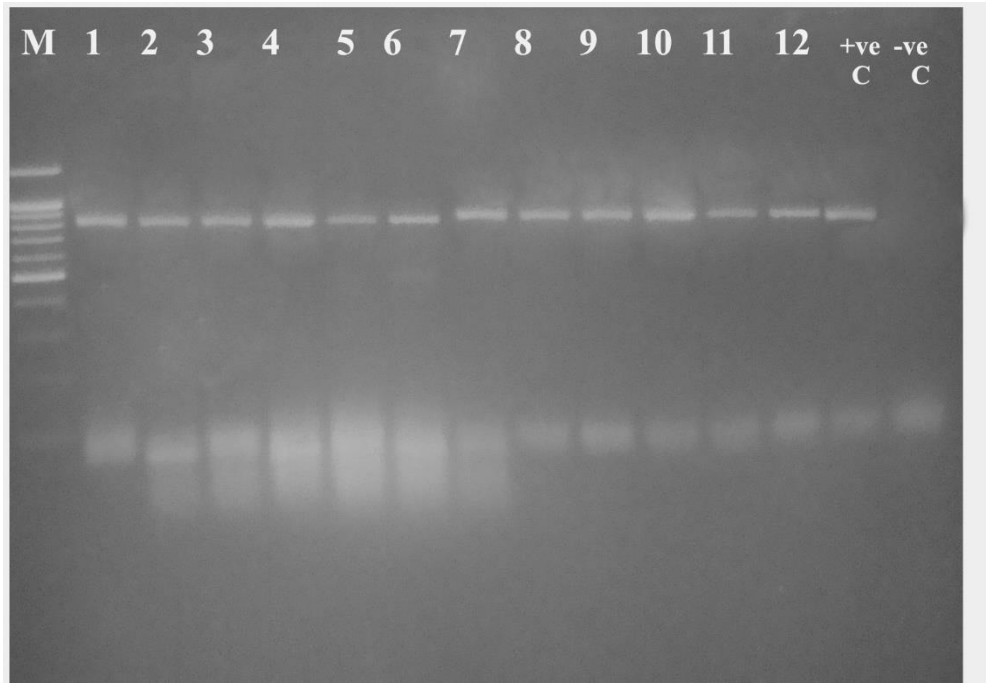

Fig.1; Lanes 1 to 12, PCR amplification using universal panfungal primer lane13; positive control, lane14; negative control; LaneM, 100 bp molecular size marker.

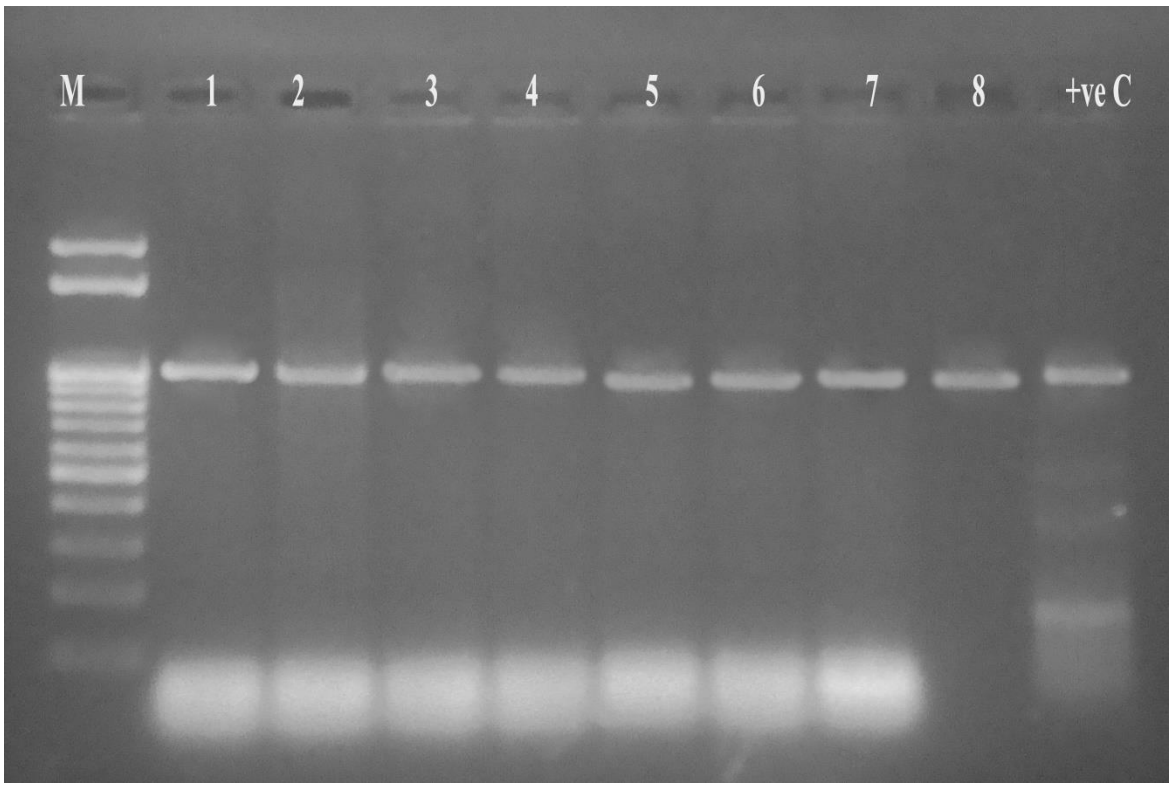

Fig.2; PCR amplification of genomic DNAs of C. albicans (lane1- 8), lane 9; positive control. Lane M; 100 bp molecular size marker

Acknowledgement

ISSN: 0974-5343
IJMST (2017), 7(2):10-17

Special thanks goes to Cairo university specialized pediatric hospital (CUSPH) clinical microbiology department for the microbiological culturs,techniques and PCR and faculty of veterinary medicine cairo university.

References:

[1] Heron M, Hoyert DL, Murphy SL, Xu J, Kochanek KD,Tejada-Vera BS. Deaths: final data for 2006. Nat Vital Stat Rep. 2009;57:1–134.

[2] Stoll BJ, Hansen N, Fanaroff AA, Wright LL, Carlo WA,Ehrenkranz RA, Lemons JA, Donovan EF, Stark AR, TysonJE, Oh W, Bauer CR, Korones SB, Shankaran S, Laptook AR, Stevenson DK, Papile LA, Poole WK. Late-onset sepsis in very low birth weight neonates: the experience of theNICHD Neonatal Research Network. Pediatrics. 2002;110:285–91.

[3] Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, WenzelRP, Edmond MB. Nosocomial bloodstream infections in UShospitals: analysis of 24, 179 cases from a prospectivenationwide surveillance study. Clin Infect Dis. 2004;39:309–17.

[4] Filioti J, Spiroglou K, Roilides E. Invasive candidiasis in pediatric intensive care patients: epidemiology, risk factors, management, and outcome. Intensive Care Med. 2007;33:1272–83.

[5] Neu N, Malik M, Lunding A, Whittier S, Alba L, Kubin C, Saiman L. Epidemiology of candidemia at a children's hospital, 2002 to 2006. Pediatr Infect Dis J. 2009;28:806–9.

[6] Saiman L, Ludington E, Pfaller M, Rangel-Frausto S, Wiblin RT, Dawson J, Blumberg HM, Patterson JE, Rinaldi M, Edwards JE, Wenzel RP, Jarvis W. Risk factors for candidemia in neonatal intensive care unit patients. Pediatr Infect Dis J. 2000;19:319–24.

[7] Chapman RL. Candida infections in the neonate. Curr. Opin. Infect. Dis. 2003; 15: 97–102.

[8] Benjamin DK Jr, De Long ER, Steinbach WJ et al. Empirical therapy for neonatal candidemia in very low birth weight infants. Pediatrics 2003; 112: 543–7.

[9] [9]Tirodker UH, Nataro JP, Smith S, et al. Detection of fungemia by polymerase chain reaction in critically ill neonates and children. J Perinatol 2003; 23:117.

[10] Pappas PG, Rex JH, Lee J et al. A prospective observational study of candidemia: Epidemiology, therapy and influences on mortality in hospitalized adult and pediatric patients. Clin. Infect. Dis. 2003; 37: 634–43.

[11] Blyth CC, Chen SC, Slavin MA, Serena C, Nguyen Q, Marriott D, Ellis D, Meyer W, Sorrel TC, AustralianCandidemia Study. Not just little adults: candidemia epidemiology,molecular characterization, and antifungal susceptibility in neonatal and pediatric patients. Pediatrics. 2009;123:1360–8.

[12] Leibovitz E ,2010 strategies for prevention of neonatal candidiasis.pediatr neonatol.53:83:9.

[13] Jeanne a. jordan,pcr Identification of Four Medically Important Candida Species by Using a Single Primer PairJOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1994, p. 2962-2967 0095-1137/94.

[14] Benjamin Jr DK, Stool BJ, Gantz MG, et al. Neonatal candidiasis: epidemiology, risk factors, and clinical judgment. Pediatrics. 2010;126:865–73.

[15] Ahmad S., Khan Z., Mustafa A. S., Khan Z. U. 2002. Seminested PCR for diagnosis of candidemia: comparison with culture, antigen detection, and biochemical methods for species identification. J. Clin. Microbiol. 40:2483–2489.

[16] Khan Z. U., Mustafa A. S. 2001. Detection of Candida species by polymerase chain reaction (PCR) in blood samples of experimentally infected mice and patients with suspected candidemia. Microbiol. Res. 156:95–102 .

[17] Tomer Avni,1, Leonard Leibovici,1 and Mical Paul2, PCR Diagnosis of Invasive Candidiasis: Systematic Review and Meta-Analysis▽ J Clin Microbiol. 2011 Feb; 49(2): 665–670.

[18] Moreira-Oliveira M. S., et al. 2005. Diagnosis of candidemia by polymerase chain reaction and blood culture: prospective study in a high-risk population and identification of variables associated with development of candidemia. Eur. J. Clin. Microbiol. Infect. Dis. 24:721–726 .

[19] Garczewska B., Dzierzanowska D. 2002. Use of molecular biology methods for diagnosing fungal infections. Med. Dosw. Mikrobiol. 54:157–165 (In Polish.)

[20] Maaroufi Y., et al. 2003. Rapid detection of Candida albicans in clinical blood samples by using a TaqMan-based PCR assay. J. Clin. Microbiol. 41:3293–3298 .

[21] Fujita, S., Y. Senda, S. Nakaguchi, and T. Hashimoto. 2001. Multiplex PCR using internal transcribed spacer 1 and 2 regions for rapid detection and identification of yeast strains. J. Clin. Microbiol. **39:**3617-3622.

[22] Hoppe., J.E., Frey., P.2001 Evaluation of Six Commercial Tests and the Germ-Tube Test for Presumptive Identification of Candida albicans. Eur J Clin Microbiol Infect Dis (1999) 18 :188–191.